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RESISTANCE OF THE PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) TO THE DEVELOPMENTAL INSECTICIDES CHLORFLUAZURON AND FLUFENOXURON

El-Tantawy[1],M.A., Amira,M.Rashad[2], Didiar, A. Ragheib[1] and H. K. Sabry[3]

[1]Plant Protection Dept.,Faculty of Agric., Zagazig Univ., Egypt.
[2]Plant Protection Res. Inst., Agric. Res. Center, Dokki, Egypt.
[3]Pests and Plant Protection Dept., National Res. Center, Dokki, Egypt.

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ABSTRACT: Development of resistance to the developmental insecticides chlorfluazuron and flufenoxuron in the newly hatched larvae of the pink bollworm, *Pectinophora gossypiella* (Saunders), laboratory and field colonies were investigated in the laboratory.

It was found that the rate of building up of resistance to both insecticides was high. Resistance ratios of chlorfluazuron in the laboratory and field colonies ranged between 3.3- to 1022.3- and 2.4- to 376.48-fold, respectively. The corresponding figures of flufenoxuron ranged between 2.7- to 489.9- and 1.1- to 209.6- fold. The rate of building up of resistance was faster in the laboratory colony than with the field one. Concerning the relative efficacy of both compounds, data show that chlorfluazuron exhibited higher capacity in the rate of building up of resistance in both colonies.

Key words: Pink bollworm, chlorfluazuron, flufenoxuron, resistance

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) is a cosmopolitan insect species of considerable economic importance. In Egypt, the cotton producers suffer from the high loss in the quality and quantity of the cotton yield.

The chitin synthesis inhibitors chlorfluazuron and flufenoxuron exhibited excellent action in inhibiting the development of many insect species (Gijswijt et al., 1979 and Lim and Khoo, 1986).

During the last two decades, obvious levels of resistance to chlorfluazuron and flufenoxuron were observed in many pests such as the diamondback moth, *Plutella xylostella* (Sinchaisri et al., 1989; Sun et al., 1990; Fahmy and Miyata, 1991; Wu et al., 1998; and Ohtsu et al., 1999) and the codling moth, *Cydia pomonella* L. (Sauphanor et al., 1998)

Although chlorfluazuron and flufenoxuron are included in the program of the chemical control of the pink bollworm in Egypt, the review of literature is free from any trials regarding development of resistance of this pest to these two insecticides. Such studies may throw some light on the possibility of using this group of chemical economically during a known period against this pest.

The aim of the present work is to detect the rate of development of resistance to the preceding two chitin synthesis inhibitors using the newly hatched larvae of the laboratory and field colonies of the pink bollworm, *P. gossypiella* under the laboratory conditions.

MATERIALS AND METHODS

Test Insects

A laboratory and field colonies of the pink bollworm, *P. gossypiella* were used. The laboratory colony was obtained from the Bollworm Research Division, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. This colony was continuously reared in the laboratory since ten years without any exposure to any pesticides. The field colony was obtained from EL-Ibrahimia region, Sharkia Governorate during 2001 -2002 cotton season by collecting the green bolls during November. The collected bolls were exposed to sun until dryness and kept in the laboratory until January. The diapaused larvae, obtained from the dried bolls, kept in convenient glass tubes (3x10 cm) closed with a piece of cotton until pupation and adult emergence. The emerged moths were sexed; each pair was transferred to a glass chimney cages covered with a muslin cloth as a suitable site for egg

deposition. The moths were fed on sucrose solution (10%).The muslin was replaced every 3 days. The eggs deposited on the muslin were transferred to convenient glass jars until hatching. To simulate nature, the newly hatched larvae were used in selection study. The colonies were reared at 27± 1 ℃ and 65-75% R.H. with complete dark all daytime. The different larval instars were fed on a semi-artificial diet (Rashad and Ammar, 1985).

Test Chemicals

a-Chlorfluazuron (Atabron EC 5%)

-**Chemical name**: 1-[3, 5-dichloro-4(3-chloro-5-trifluoromethyl-2-pyridyloxy) phenyl]-3-(2, 6-difluorobenzoyl) urea.

b-Flufenoxuron (Cascade EC 10%)

-**Chemical name**: 1-[4-(2-chloro-α, α, α- trifluoro-p-tolyloxy)-2-fluorophenyl]-3-(2,6difluorobenzoyl) urea

Bioassay

Stock solution of each formulation was achieved by mixing the formulation product with distilled water. Different stock solutions were prepared to be used in each generation depending on the mortality percentages obtained during the preceding generation of pressure as well as the source of the used colony (i.e., laboratory and field colony). With each generation of selection pressure at least four concentrations were used to avoid losing the colony with using higher concentration during the next generation.

The amount of the used formulation was added to 50gr. fresh prepared diet. This amount of treated diet was divided into three volumes (Ca. 16gr.). Each one was poured into a convenient petri dish (12cm in diameter). Fourty healthy newly hatched larvae, starved for 6hrs, were gently transferred to each petri dish using a soft brush. With this way three replicates were used for each concentration. Similar number of larvae were transferred to pesticide - free diet and considered as the control treatment.

In each selected generation, the highest concentration used in the preceding generation was used with other two higher concentrations. The range between the used concentrations of each generation depended on the viability of the offspring of the preceding generation. Wide ranges of concentrations were used to detect the median lethal

concentration (LC_{50}) during the onset of detecting the susceptibility of each colony as well as during assessing the fold of resistance in all generation during selection. Concentrations ranged between 36.27 to 17770.02 and 94.18 to 17888.73 ppm with flufenoxuron laboratory and field colonies, respectively, and 9.24 to 7545.27 and 52.25 to 17888.73 ppm with chlorfluazuron laboratory and field colonies, respectively.

The dishes were maintained in an incubator at a temperature of 27± 1 ºC and 65-75% R.H. with a complete dark all daytime. After one hour, from exposing the first instar larvae to the insecticide-contaminated diet or the free one, the larvae were transferred individually into clean and sterile glass tubes (2x7cm) each containing a small piece of untreated diet; each tube contained one alive larva. After 5, 11, and 17days (after the first two molts) all tubes were inspected for estimating the mortality percentages. The LC_{50} as well as resistance ratios were determined after each generation. The resistance ratios were obtained from dividing the LC_{50} for the resistance colony on the corresponding value of the susceptible colony. LC_{50} and slope values and their confidence limit were obtained from Propan Program Soft Ware Computer.

RESULTS AND DISCUSSION

Data presented in Table 1 and Figure 1 show that the LC_{50} values of chlorfluazuron obtained from the exposure of the newly hatched larvae of the pink bollworm, *P. gossypiella* to these insecticides were sharply increased during the majority of the experimental period (20 generations). LC_{50} values ranged between 9.24 and 9445.67 ppm. These figures show that high levels of the susceptible and tolerant individuals greatly suffered from the action of chlorfluazuron which resulted in obvious mortality percentages. Such sieving of the preceding individuals gave chance to the secured ones, i.e. the resistant individuals and to some extent the vigor tolerant ones to increase progressively from generation to other. Resistance ratios (RR) show that LC_{50} values progressively increased during the first five generations then sharply decreased in the next generation. The same phenomenon took place during the eighth generation. During the next twelve generations

Table 1: Buildup of resistance in newly hatched larvae of pink bollworm, *P gossypiella* (laboratory colony) against chlorfluazuron

Generations	LC_{50} and Confidence limits	Slope	Resistance ratios
F_0	9.24(7.78 – 10.98)	1.75 ± 0.21	---
F_1	30.68(26.71 – 35.23)	2.29 ± 0.32	3.3
F_2	104.56(94.10 – 116.17)	2.94 ± 0.32	11.3
F_3	260.92(238.14 – 258.88)	3.61 ± 0.43	28.2
F_4	693.59(653.45 – 736.19)	5.04 ± 0.60	75.1
F_5	1225.67(1137.13 – 1321.11)	4.22 ± 0.61	132.6
F_6	100.27(90.46 – 111.15)	3.06 ± 0.32	10.9
F_7	1183.10(1122.97 – 1246.45)	6.51 ± 0.70	128.1
F_8	94.27(84.10 – 105.65)	2.80 ± 0.31	10.2
F_9	775.39(656.71 – 915.51)	1.99 ± 0.36	83.9
F_{10}	3116.29(2776.17 – 3515.81)	2.83 ± 0.37	337.3
F_{11}	4913.61(4423.45 – 5458.08)	2.91 ± 0.32	531.8
F_{12}	9445.67(8850.79 – 10080.51)	4.71 ± 0.61	1022.3
F_{13}	5756.98(5499.20 – 6026.85)	6.79 ± 0.79	623.1
F_{14}	8314.56(8042.30 – 8596.04)	8.97 ± 1.18	899.8
F_{15}	3274.30(3005.69 – 3566.92)	3.48 ± 0.46	354.4
F_{16}	5313.58(4981.20 – 5668.14)	4.64 ± 0.62	575.1
F_{17}	2520.72(2257.41 – 2859.59)	2.57 ± 0.32	272.8
F_{18}	3016.35(2799.99 – 3249.42)	4.22 ± 0.47	326.4
F_{19}	4863.41(4088.70 – 4989.11)	3.17 ± 0.48	526.3
F_{20}	7545.27(7167.27 – 7942.70)	6.21 ± 0.82	816.6

Table 2: Buildup of resistance in newly hatched larvae of pink bollworm, *P. gossypiella* (field colony) against chlorfluazuron

Generations	LC_{50} and Confidence limits	Slope	Resistance ratios
F_0	52.25 (44.63 – 61.17)	2.05±0.22	---
F_1	126.62 (112.86 – 142.06)	2.65± 0.31	2.4
F_2	243.62 (218.86 – 271.18)	2.84± 0.32	4.7
F_3	909.19 (862.13 – 958.83)	6.02± 0.57	17.4
F_4	879.11 (799.58 – 913.41)	6.01± 0.18	16.8
F_5	6171.78 (5866.73 – 6492.69)	6.06± 0.66	118.1
F_6	9301.29 (8997.54 – 9615.30)	9.19± 1.10	178.0
F_7	8228.55 (7982.32 – 8482.38)	10.03± 1.20	157.5
F_8	10027.23 (9742.04 – 10320.77)	10.87± 1.46	191.9
F_9	5212.83 (4904.37 – 5540.69)	5.00± 0.63	99.7
F_{10}	2545.69 (2198.19 – 2948.11)	2.10± 0.30	48.7
F_{11}	4206.02 (3864.15 – 4578.13)	4.14± 0.50	80.5
F_{12}	19670.97 (19126.32 – 20231.13)	14.78± 1.94	376.48
F_{13}	15093.06 (14840.11 – 15350.33)	18.53±2.22	288.86
F_{14}	16552.08 (16302.55 – 16805.42)	19.82± 2.36	316.79
F_{15}	17888.73 (17572.40 – 18210.75)	18.37± 2.58	342.37

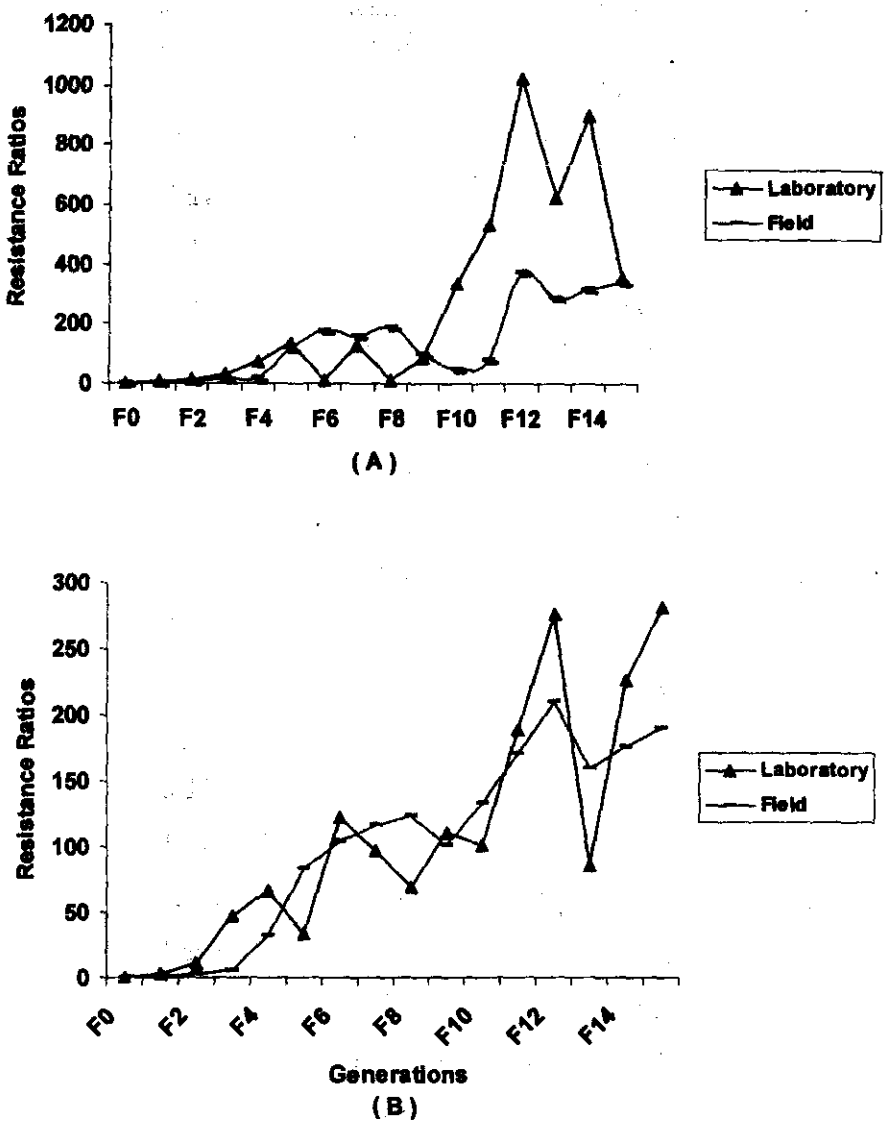

Fig.1: Resistance of the laboratory and field colonies of the pink boilworm, *P. gossypiella* to (A) chlorfluazuron and (B) flufenoxuron

Table 3: Buildup of resistance in newly hatched larvae of pink bollworm, *P. gossypiella* (laboratory colony) against flufenoxuron

Generations	LC_{50} and Confidence limits	Slope	Resistance ratios
F_0	36.27(30.33 – 43.36)	1.68± 0.21	---
F_1	96.20(82.76 – 111.82)	2.16± 0.22	2.7
F_2	402.48(366.94 – 441.47)	3.50± 0.34	11.1
F_3	1707.61(1629.93 – 1788.99)	6.65± 0.83	47.1
F_4	2433.24(2361.97 – 2506.65)	11.50± 1.37	67.1
F_5	1208.79(1098.44 – 1330.24)	3.35± 0.59	33.3
F_6	4409.81(4167.69 – 4665.98)	5.35± 0.63	121.6
F_7	3530.49(3276.00 – 3804.75)	4.04± 0.48	97.4
F_8	2512.31(2205.47 – 2861.84)	2.31± 0.31	69.3
F_9	4039.52(3699.50 – 4410.79)	3.43± 0.40	111.4
F_{10}	3669.84(3386.69 – 3972.32)	3.85± 0.47	101.1
F_{11}	6844.75(6284.36 – 7455.10)	3.82± 0.58	188.7
F_{12}	10048.50(9714.72 – 10393.75)	9.25± 1.09	277.0
F_{13}	3110.43(2799.11 – 3815.81)	10.09± 1.11	85.8
F_{14}	8220.77(7931.95 – 8520.10)	8.38± 1.17	226.7
F_{15}	10243.87(10020.57 – 10472.14)	14.04± 1.53	282.4
F_{16}	11133.16(10874.43 – 11398.04)	13.14± 1.63	306.9
F_{17}	11250.68(10931.06 – 11579.60)	10.64± 1.32	310.2
F_{18}	13648.99(13384.10 – 13919.14)	15.39± 1.90	376.3
F_{19}	13584.34(13315.60 – 13858.50)	14.96± 1.90	374.5
F_{20}	17770.02(17408.90 – 18138.60)	15.36± 1.60	489.9

Table 4: Buildup of resistance in newly hatched larvae of pink bollworm, *P. gossypiella* (field colony) against flufenoxuron

Generations	LC_{50} and Confidence limits	Slope	Resistance ratios
F_0	94.18(80.99 – 109.50)	2.13± 0.22	---
F_1	107.78(97.62 – 118.99)	3.14± 0.32	1.1
F_2	212.44(188.05 – 239.99)	2.47± 0.31	2.3
F_3	579.78(543.44 – 618.54)	4.75± 0.50	6.2
F_4	3064.59(2651.64 – 3541.85)	2.12± 0.24	32.5
F_5	7854.29(7533.06 – 8189.20)	7.33± 0.86	83.4
F_6	9811.79(9535.91 – 10095.65)	10.94± 1.14	104.2
F_7	10972.61(10710.81 – 11240.82)	13.30± 1.64	116.5
F_8	11695.26(11409.33 – 11988.35)	14.28± 1.81	124.2
F_9	9581.74(9237.89 – 9938.38)	8.13± 1.06	101.7
F_{10}	12545.48(12085.24 – 13.23.25)	10.49± 1.63	133.2
F_{11}	16050.38(15662.13 – 16448.27)	15.09± 1.46	170.4
F_{12}	19737.28(19214.18 – 20274.63)	15.79± 2.01	209.6
F_{13}	15093.06(14840.10 – 15350.33)	18.53± 2.22	160.3
F_{14}	16552.07(16302.55 – 16805.42)	19.82± 2.36	175.7
F_{15}	17888.73(17572.40 – 18210.75)	18.37± 2.58	189.9

the fluctuation in the LC_{50} values from generation to another was not magnitude as with the sixth and eighth generations. Fluctuations in the LC_{50} and slope values may be due to the irregularity in the withstanding criterion of some tolerant individuals which refers to the heterogeneity of the laboratory individuals which may constitute unavoided level of such individuals.

As expected, the rate of building up of resistance in the laboratory colony was higher than that with the field colony (Table 2 and figure 1).Resistant ratios ranged between 3.3-and 1022.3 – fold in the laboratory colony compared with 2.4 and 376.48-fold in the field one. Such difference between the two colonies may be due to the lower level of susceptible individuals in the field colony due to continuous exposure to conventional and developmental insecticides during control programs. This may reflect the lower fluctuations in the LC_{50} values of the field colony from generation to other than those of the laboratory one. With the exception of the ninth, tenth and eleventh generations, there was progressive increase in the LC_{50} values during the whole pressure period. Such decrease in the efficiency of chlorfluazuron during these three generations were, however, lower than those previously mentioned during the sixth and eighth generations of the laboratory colony.

Data summarized in Tables 3 and 4 as well as Figure 1 show that the rate of building up of resistance to flufenoxuron in the laboratory and field colonies was lower than with chlorfluazuron. Resistance ratios to flufenoxuron ranged between 2.7- to 489.9- and 1.1- to 209.6-fold with the laboratory and field colony, respectively. The corresponding ranges in chlorfluazuron were 3.3- to 1022.3- and 2.4- to 376.48-fold. In the fifteenth generation resistance ratios were 282.4- and 189.9- fold in the laboratory and field colonies, respectively. These figures reflect the capacity of flufenoxuron in eliminating the susceptible and tolerant individuals which occupied higher level in the laboratory colony. Based on this, the development of resistance in the laboratory colony was faster than the field one. It is worth to note that the obvious fluctuation in building up of resistance in case of chlorfluazuron was, however, slight with flufenoxuron.

Data presented in Tables 1, 2, 3, and 4 clearly showed that

chlorfluazuron (Atabron 5% EC) was more toxic than flufenoxuron (Cascade 10 % EC). The LC_{50} values of the former compound, initially used with the parents, were 9.24 and 52.25 ppm in the laboratory and field colony, respectively. The corresponding figures of flufenoxuron were 36.27 and 94.18 ppm. The superiority of chlorfluazuron may be due to the (3-chloro-5-trifluoromethyl-2pyridyloxy) phenyl moiety. The higher rate of developing resistance to chlorfluazuron in the laboratory colony of diamondback moth, *Plutella xylostella* compared with the field one was also observed by Fauziah et al. (1990).

Fahmy and Miyata (1991) showed that 80-fold resistance to chlorfluazuron in the diamondback moth took place after three selection generations. The authors suggested that the resistance gene (s) has, in large measure been kept in the population.

The fluctuation in the LC_{50} values from generation to another was also observed by Cheng and Kao (1998); Ohtsu et al. (1999) working on the resistance of the diamondback moth, *Plutella xylostella* to chlorfluazuron.

Resistance of the diamondback moth to the other class of developmental insecticides, i.e., the juvenoides was also noted (Kobayashi et al., 1990).

Wang et al. (2002) detect 25-fold of resistance to chlorfluazuron in *Spodoptera exigua*. On the other hand, low levels of resistance to chlorfluazuron were attained with other insect species (Yuan et al., 2000; Hao et al., 2002; and Ishaaya et al., 2003).

In conclusion, the obtained results show that the rate of building up of resistance to the two chitin synthesis inhibitors chlorfluazuron (Atabron EC 5 %) and flufenoxuron (Cascade EC 10 %) is very high. Thus using these two developmental insecticides for more one application during the same season is not recommended. The exposure of the used toxicants to environmental degradation under field conditions may add extra limitation.

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مقاومة دودة اللوز القرنفلية لمبيدي الكلورفلوازيورون و الفلوفينوكسيرون

مصطفي عبد الحفيظ الطنطاوي[1] - أميرة محمد رشاد[2] - ديدير أحمد راغب[1]
القذافي حسن صبري[3]
[1]قسم وقاية النبات- كلية الزراعة- جامعة الزقازيق- الزقازيق- مصر
[2]معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي- جيزة- مصر
[3]قسم آفات ووقاية النبات- المركز القومي للبحوث- الدقي- جيزة - مصر

درس تطور مستويات المقاومة لمجتمعين من دودة اللوز القرنفلية (احدهما معملية والآخري حقلية) عند تعريض يرقات العمر الأول حديث الفقس لمركبين من المركبات المثبطة لتخليق الكيتين هما الكلورفلوازيرون(أتابرون ٥ % مركز قابل للإستحلاب) و الفلوفينوكسيرون (كاسكيد ١٠ % مركز قابل للإستحلاب). عرض كلا المجتمعين لضغط إنتخابي لمدة عدة أجيال مختلفة.

أظهرت النتائج أن بناء صفة المقاومة لكلا المركبين كان مرتفع. نسب المقاومة للكلورفلوازيرون تراوحت بين 3.3 إلى 1022.3 و 2.4 إلى 376.48 ضعف في السلالة المعملية و الحقلية علي التوالي. وتراوحت نسب المقاومة بين 2.7 إلى 489.9 و 1.1 إلى 209.6 ضعف باستخدام المركب الآخر (الفلوفينوكسيرون). معدل بناء المقاومة في السلالة المعملية كان سريعا مقارنة بالسلالة الحقلية. أدي تعرض اليرقات لمركب الكلورفلوازيرون الي الحصول علي مستوي عالي من المقاومة في كلا السلالتين مقارنة بمركب الفلوفينوكسيروت.